Growth Plate Cartilage Formation and Resorption Are Differentially Depressed in Growth Retarded Uremic Rats

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Abstract. To characterize the modifications of growth plate in individuals with growth impairment secondary to chronic renal failure, young rats were made uremic by subtotal nephrectomy (NX) and, after 14 d, their tibial growth plates were studied and compared with those of sham-operated rats fed ad libitum (SAL) or pair-fed with NX (SPF). NX rats were growth retarded and severely uremic. Growth plate height (mean ± SD) was much greater (P < 0.05) in NX (868.4 ± 85.4 μm) than SAL (570.1 ± 93.5 μm) and SPF (551.9 ± 99.7 μm) rats as a result of a higher (P < 0.05) hypertrophic zone (661.0 ± 89.7 μm) versus 362.8 ± 71.6 and 353.0 ± 93.9 μm, respectively). The increased size of the growth plate was associated with a greater number of chondrocytes and modifications in their structure, particularly in the hypertrophic zone adjacent to bone. In this zone, chondrocytes of NX animals were significantly (P < 0.05) smaller (12080.4 ± 6158.3 μm³) than those of SAL (16302.8 ± 1483.4 μm³) and SPF (14465.8 ± 1521.0 μm³ and 353.0 ± 1.8 μm³). The interface between the growth plate cartilage and the metaphyseal bone appeared markedly irregular in NX rats. Kinetics of chondrocytes was also modified (P < 0.05) in the NX rats, which had lower cell turnover per column per day (5.4 ± 0.9), longer duration of hypertrophic phase (89.0 ± 15.2 h), and reduced cellular advance velocity (7.4 ± 2.2 μm/h) compared with SAL (8.0 ± 1.6, 32.1 ± 6.7 h, and 11.3 ± 2.7 μm/h) and SPF (7.2 ± 1.1, 34.8 ± 5.1 h, and 10.1 ± 2.5 μm/h). Cell proliferation was no different among the three groups. Because the growth plates of SPF and SAL rats were substantially not different, modifications observed in the NX rats cannot be attributed to the nutritional deficit associated with renal failure. These findings indicate that chronic renal failure depresses both the activity of the growth plate cartilage by altering chondrocyte hypertrophy and the replacement of cartilage by bone at the metaphyseal end. The two processes are differentially depressed since cartilage resorption is more severely lowered than cartilage enlargement and this leads to an accumulation of cartilage at the hypertrophic zone.

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Growth failure is a common manifestation of advanced chronic renal insufficiency in children. More than 50% of pediatric patients show growth velocities below the third percentile for bone age at the beginning of dialysis treatment, and only in a minority is growth velocity above the 50th percentile (1,2). Furthermore, normal growth rate is usually not restored during chronic dialysis or even after successful renal transplantation (3–6). As a result, growth retardation has probably become the single most important problem in the clinical management of children with chronic renal failure.

Pathogenesis of growth retardation in chronic renal failure is not fully understood (7,8). Although the origin of growth impairment may be multifactorial, nutritional and hormonal disorders usually play a major role, with insensitivity to endogenous growth hormone (GH) considered the main causal factor (9,10). Much effort has been devoted in recent years to characterize the alterations induced by renal failure in GH metabolism, either at the pituitary (11–14), hepatic (15,16), or peripheral level (17–19). However, since the study by Mehls et al. (20) in 1977, little progress has been made in the understanding of the effect of uremia on the structure and dynamics of epiphyseal growth plate, which is the effector organ of longitudinal growth.

Longitudinal bone growth results from progressive replacement of growth plate cartilage by osseous tissue at the metaphyseal ends. Physiologically, the rate and extent of growth for a given growth plate is determined by a combination of chondrocyte proliferation, matrix production, and increase of chondrocyte volume (21). The few studies reporting data on the characteristics of epiphyseal growth plate in uremic rats have been restricted mostly to the measurement of bulk parameters such as growth plate height and daily longitudinal growth rate of bone, as assessed by fluorescence labeling (20,22–26). However, little is known about the effects of uremia on growth plate chondrocyte activity. By using a methodologic approach similar to that described by Hunziker et al. (27,28), the present study reports a detailed histomorphometric analysis of the
alterations found in the epiphyseal growth plate of uremic rats and provides new information on the activity of chondrocytes in chronic failure.

Materials and Methods

Animals and Experimental Protocol

Male Sprague Dawley rats, 22 d old, were housed in individual cages and fed a standard 23.9% protein rat chow (AO3, Panlab SL, Barcelona, Spain). After 3 d of adaptation to the experimental area, the animals were classified in three groups of five rats each: 5/6 nephrectomized (NX), sham-operated fed ad libitum (SAL), and sham-operated pair-fed with NX (SPF). At the beginning of the experiment, rat weights ranged from 70 to 90 g with a mean weight of 82 g. The mean weight of all three groups of rats was not different from one another at this time.

Subtotal nephrectomy or sham operation was performed in two stages, on day 0 and day 4, as reported previously (11). SPF rats were given the same amount of food consumed by NX animals the day before.

Rats’ body growth was assessed by weight and length gained between day 11 and day 18. Rats and food consumption were weighed daily with an electronic balance Ohaus GT 2001 (Ohaus Scale Corp., Florham Park, NJ). Nose to tail length was measured under anesthesia on days 0, 11, and 18. On day 18, rats were sacrificed by exsanguination under anesthesia. At that moment, blood was obtained for measuring serum concentrations of urea nitrogen, with an autoanalyzer Kodak EktachemR (Eastman Kodak, Rochester, NY) and both tibiae were removed. All animals received intraperitoneal injections of calcein (Sigma, St. Louis, MO) at a dose of 15 mg/kg body wt and 5-bromo-2'-deoxy-uridine (BrdU) (Sigma) at a dose of 100 mg/kg body wt, on day 14 and 1 h before sacrifice, respectively.

Tissue Collection and Processing

After removal, tibia lengths and their frontal and sagittal diameters at the level of the upper growth plates were measured with a sliding mechanical caliper (accuracy 10 μm). Frontal and sagittal diameters were used for estimation of the growth plate area perpendicular to the long axis according to the ellipse formula (29). Blocks from proximal tibial growth plate were chosen by a systematic random-sampling process, as reported by Cruz-Orive and Hunziker (29). Briefly, each growth plate was dissected with the aid of a stereomicroscope into 10 prismatic blocks. Care was taken that sections were oriented parallel to the longitudinal axis of tibiae (parallel to the plane of anisotropy). Tissue blocks were numbered from 1 to 10, and five of them were randomly chosen in each tibia. The first two blocks were used for measuring the rate of longitudinal bone growth, the next two blocks were used for histomorphometric analysis, and the last one was used for immunocytochemical identification of proliferating chondrocytes.

Tissue blocks for determination of longitudinal growth rate were fixed in a mixture of 0.5% glutaraldehyde and 4% paraformaldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, for 5 h at 4°C. After washing in buffer, samples were dehydrated in ascending concentrations of acetone and embedded in Durkupan-ACM.

Embedded tissues were cut on a Reichert Ultracut E ultramicrotome parallel to the tibial vertical axis, with the section angle randomly oriented relative to the horizontal plane for each block. The mean thickness of the sections was 1.08 ± 0.04 (SD) μm.

Determination of Longitudinal Bone Growth Rate

Sections were examined under a Leitz incident light fluorescence microscope, and the distance between the zone of vascular invasion and the proximal part of the calcein label was measured with an eyepiece micrometer (30). Measurements were obtained at three unbiasedly determined locations on each of four sections per animal, and the mean value of these measurements divided by 4 (days) was considered the longitudinal bone growth per day in each animal. The volume of newly formed bone per day was estimated by multiplying this value by the growth plate area (31).

Immunocytochemical Identification of Proliferating Chondrocytes

Semithin sections were etched in 50% sodium ethoxide, pH 12.5, for 15 min. Etching resulted in both deplastification of the tissues and denaturing of the nuclear DNA. After etching, sections were passed through graded alcohols, washed in Tris buffer (0.1 M, pH 7.4), and incubated overnight with an anti-BrdU monoclonal antibody (1:20; Dakopatts, Glostrup, Denmark) in a moist chamber at 4°C, followed by incubation with biotinylated antimouse IgG (1:100, 60 min at room temperature; Biomeda Corp., Foster City, CA) and streptavidin-peroxidase complex (45 min at room temperature). The final reaction product was developed by incubation with a solution containing 0.66 mM 3,3'-diaminobenzidine and 2 mM H2O2 in 50 mM Tris-HCl, pH 7.6. Preparations were lightly counterstained with Gill’s hematoxylin and mounted. Proliferative activity was quantified by the estimation of the labeling index (LI), which was defined as the percentage of BrdU-labeled cells within the proliferative stratum.

Histomorphometry

Sections were stained with toluidine blue and photographed by light microscopy at a first low magnification (×230). On these prints, growth plates were divided into stem cell, proliferating, and hypertrophic zones according to morphologic criteria (29). The hypertrophic zone was arithmetically subdivided into an upper (H1) and lower (H2) half. Boundaries of zones were marked on the paper prints. The height of the proliferative zone was calculated as the mean of three different measurements performed at three locations randomly chosen on each section. The heights of the growth plate and its different zones were estimated by point counting from the height of the proliferative zone.

The volume of the growth plate and the volume of the different zones were estimated by multiplying the growth plate area by the mean growth plate height and the mean height of the different zones, respectively.

From each section, two quadrants from the proliferating zone and one from the hypertrophic zone were subsampled, photographed, and printed on paper with a final magnification factor of ×700. The boundaries of the zones were copied from those on low magnification prints. The following stereologic parameters were determined within each zone: volume fraction of chondrocytes, numerical density of chondrocytes, total number of chondrocytes, mean chondrocyte vol-
The volume fraction of chondrocytes was estimated by using a transparent screen with a square point lattice spacing of 21 mm (equivalent to 30 μm) and counting the number of intersections on chondrocytes and on matrix. The volume fraction of chondrocytes in each stratum was calculated by dividing the total number of points over chondrocytes by the total number of points. Total volume of chondrocytes was then estimated by multiplying this value by the volume of the stratum. Total volume of matrix was estimated by deducting the volume of chondrocytes from the volume of the stratum. The numerical density and total number of chondrocytes were estimated using the disector method (29) on the first and the fifth sections of a series of consecutive sections. The number of chondrocytes present in the first section but no longer apparent in the fifth divided by the volume of the disector (reference area 1837 μm² per the height of the disector −4.32 μm) gives an estimate of the number of chondrocytes per unit of reference volume. The total number of chondrocytes was calculated by multiplying this value by the volume of the stratum. The mean chondrocyte volume and mean matrix volume per chondrocyte were estimated by dividing the total volume of chondrocytes and the total volume of matrix by the total number of chondrocytes, respectively. The mean projected horizontal diameter of a chondrocyte was defined as the caliper diameter measured perpendicular to the long axis of the bone. The mean chondrocyte height was estimated from the mean profile height. It was defined as the length of the intercept bisecting the cell profile measured parallel to the long axis of the bone. Estimators of cell diameters based on shape models were not adequate in the present study, since NX animals showed alterations in the shape of hypertrophic chondrocytes and were not adjusted to a super-egg model. Then, the mean diameters were obtained by direct measurements of the vertical and horizontal axes of 500 chondrocyte profiles cut centrally (through the nucleus) per animal (27). The number of cells in a vertical cell column was obtained by dividing the height of the stratum by the mean chondrocyte height (29). Due to the bias involved in its estimation, this parameter was also determined directly by counting the number of cells within complete column profiles in the section plane (27). Both procedures yielded similar results. Finally, the growth fraction was estimated by dividing in a column the number of chondrocytes at the proliferative zone by the total number of chondrocytes (27).

**Statistical Analyses**

Values of each group are given as mean ± SEM. For the growth plate data, a mean value for each of the tested parameters was calculated on a per-animal basis in the group. Then, each animal was considered a sample for statistical purposes. The data obtained were shown to follow a normal distribution with homogeneity of variances and independence. Comparison among the three groups was performed by ANOVA, using a significance level of 95%, followed by the Newman–Keuls multiple range test.

**Results**

Severe renal failure was induced in NX rats as confirmed by serum concentrations of urea nitrogen levels 6 to 7 times higher than those found in SAL and SPF animals (Table 1). Cumulative food intake of NX rats was 66% of that of SAL and, according to the experimental protocol, almost identical to that of SPF animals. NX animals were growth-etarded as shown by low gains in body weight and length and less tibial length and longitudinal growth rate than control animals (Figure 1).

Under the light microscope, the growth plates appeared bigger and more irregular in NX than SPF and SAL groups (Figures 1 and 2). Stereologic parameters describing the features of growth plate and chondrocytes are given in Tables 2 and 3. The height of the entire growth plate was markedly greater in NX than control rats. Growth plate enlargement was

**Table 1. Serum urea nitrogen, cumulative food intake, and body and bone growth data in the three groups of rats**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SAL (mg/dl)</th>
<th>SPF (mg/dl)</th>
<th>NX (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUN</td>
<td>17.4 ± 2.7</td>
<td>14.4 ± 2.2</td>
<td>105.8 ± 46.1b,c</td>
</tr>
<tr>
<td>Food intake (g)</td>
<td>168.7 ± 26.2</td>
<td>107.9 ± 13.6b</td>
<td>111.0 ± 17.2b</td>
</tr>
<tr>
<td>Weight gain (g)</td>
<td>60.5 ± 11.2</td>
<td>23.2 ± 14.3b</td>
<td>42.0 ± 11.9b,c</td>
</tr>
<tr>
<td>Length gain (cm)</td>
<td>3.8 ± 0.7</td>
<td>3.5 ± 0.4</td>
<td>2.3 ± 0.9b,c</td>
</tr>
<tr>
<td>Tibial length (mm)</td>
<td>31.9 ± 0.9</td>
<td>31.6 ± 0.4</td>
<td>29.7 ± 0.7b,c</td>
</tr>
<tr>
<td>Tibial width at GP level (mm²)</td>
<td>39.8 ± 4.2</td>
<td>27.7 ± 3.1b</td>
<td>37.2 ± 2.2c</td>
</tr>
<tr>
<td>Longitudinal growth rate (μm/d)</td>
<td>304.0 ± 13.6</td>
<td>266.0 ± 19.9b</td>
<td>189.6 ± 21.5b,c</td>
</tr>
<tr>
<td>Volume of formed bone (mm³/d)</td>
<td>12.1 ± 1.8</td>
<td>7.4 ± 1.3b</td>
<td>7.1 ± 1.1b</td>
</tr>
</tbody>
</table>

aData are given as mean ± SD. SAL, control rats fed ad libitum; SPF, control rats pair-fed with NX; NX, 5/6 nephrectomized rats; SUN, serum urea nitrogen; GP, growth plate.

bP < 0.05 compared with SAL.

cP < 0.05 compared with SPF.
Figure 1. Parallel sections of the proximal tibial growth plates of sham-operated rats fed ad libitum (SAL) (a) and 5/6 nephrectomized (NX) rats (b) viewed by bright-field microscopy after toluidine blue staining (left) and by incident-light fluorescence microscopy (right). The distance between the lower border of the growth plate and the fluorochrome-labeled front appears clearly longer in SAL than in NX rats. Figure 1C is a higher magnification of the proximal end point of the calcein front in the metaphysis of a control rat. Magnification: ×25 in A and B; ×110 in C.

Figure 2. Light micrographs of vertical semithin sections of the proximal tibial growth plate of SAL rats (a), sham-operated rats pair-fed with NX (b), and NX rats (c). Growth plate in NX rats appears greater than in any of the control groups. Toluidine blue stain. Magnification, ×105.
mainly due to an increased height of the hypertrophic zone, and this resulted from a greater number of chondrocytes in this zone rather than an increase in cell size or extracellular matrix. Moreover, the chondrocytes of the lower half of the hypertrophic zone were smaller (less volume) and shorter (less height) in NX animals. Interestingly, in SAL rats chondrocyte volume and profile height increased by 86 and 23% from the upper to the lower hypertrophic layer, whereas in NX rats these increases were reduced to 36 and 10%, respectively. By contrast, the proliferative layer showed little variation in NX rats compared with SPF and SAL rats. There were no differences in the height or the morphologic features of the chondrocytes in this zone. Furthermore, the distribution pattern of BrdU-labeled cells was not modified in NX rats (Figure 3).

The interface between the growth plate cartilage and the metaphyseal bone appeared markedly irregular in NX rats, mainly as a result of flattened hypertrophic chondrocytes extending through the zone of the primary spongiosa (Figure 4, a and b). Within the metaphyseal bone, chondrocytes were well preserved and located (either isolated or in small clusters) in close proximity to osteoblasts and capillary vessels (Figure 4c). In addition, the pattern of the invading capillary sprouts found in control animals—a single capillary profile per terminal lacunar compartment—was clearly disrupted in NX rats. As a result, the vascular front could be hardly recognized at the light microscope in these animals.

Modifications of chondrocyte phenotype were accompanied by changes in chondrocyte kinetics (Table 4). The BrdU-labeling index and the columnar growth fraction, as estimators of cell proliferation, were no different among the three groups of animals. However, the cell turnover per column was significantly impaired by uremia, since seven to eight cells left the

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**Table 2.** Stereologic estimators of the upper tibial growth plate in the three groups of rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SAL</th>
<th>SPF</th>
<th>NX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth plate height (μm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>global</td>
<td>570.1 ± 93.5</td>
<td>551.9 ± 99.7</td>
<td>868.4 ± 85.4</td>
</tr>
<tr>
<td>resting zone</td>
<td>42.8 ± 19.5</td>
<td>27.6 ± 3.1</td>
<td>27.7 ± 7.6</td>
</tr>
<tr>
<td>proliferating zone</td>
<td>164.5 ± 10.7</td>
<td>171.3 ± 10.7</td>
<td>180.2 ± 22.1</td>
</tr>
<tr>
<td>hypertrophic zone</td>
<td>362.8 ± 71.6</td>
<td>353.0 ± 93.9</td>
<td>661.0 ± 89.7</td>
</tr>
<tr>
<td>Growth plate volume (mm³)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>global</td>
<td>22.6 ± 4.2</td>
<td>15.3 ± 4.0</td>
<td>32.3 ± 3.8</td>
</tr>
<tr>
<td>resting zone</td>
<td>1.7 ± 0.7</td>
<td>0.8 ± 0.2</td>
<td>1.0 ± 0.4</td>
</tr>
<tr>
<td>proliferating zone</td>
<td>6.5 ± 0.7</td>
<td>4.7 ± 1.8</td>
<td>6.7 ± 1.1</td>
</tr>
<tr>
<td>hypertrophic zone</td>
<td>14.4 ± 3.1</td>
<td>9.8 ± 3.6</td>
<td>24.6 ± 3.8</td>
</tr>
<tr>
<td>Volume fraction of chondrocytes (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>resting zone</td>
<td>21.0 ± 6.0</td>
<td>16.2 ± 2.9</td>
<td>21.0 ± 3.6</td>
</tr>
<tr>
<td>proliferating zone</td>
<td>34.4 ± 6.0</td>
<td>27.8 ± 2.9</td>
<td>32.7 ± 4.5</td>
</tr>
<tr>
<td>upper hypertrophic zone</td>
<td>54.5 ± 4.9</td>
<td>53.1 ± 8.9</td>
<td>51.6 ± 7.2</td>
</tr>
<tr>
<td>lower hypertrophic zone</td>
<td>67.0 ± 3.6</td>
<td>61.2 ± 12.3</td>
<td>62.7 ± 8.7</td>
</tr>
<tr>
<td>Numerical density of chondrocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(no. of cells × 10³/mm³ of tissue)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>resting zone</td>
<td>196.5 ± 36.9</td>
<td>170.0 ± 39.8</td>
<td>220.0 ± 23.0</td>
</tr>
<tr>
<td>proliferating zone</td>
<td>264.6 ± 30.2</td>
<td>223.7 ± 23.0</td>
<td>253.6 ± 22.6</td>
</tr>
<tr>
<td>upper hypertrophic zone</td>
<td>62.3 ± 5.4</td>
<td>59.1 ± 10.1</td>
<td>57.9 ± 8.3</td>
</tr>
<tr>
<td>lower hypertrophic zone</td>
<td>41.1 ± 5.1</td>
<td>42.3 ± 6.9</td>
<td>51.9 ± 7.4</td>
</tr>
<tr>
<td>Total number of chondrocytes (×10³)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>resting zone</td>
<td>334.1 ± 65.5</td>
<td>136.0 ± 32.9</td>
<td>220.0 ± 23.0</td>
</tr>
<tr>
<td>proliferating zone</td>
<td>1719.9 ± 43.2</td>
<td>1051.4 ± 114.9</td>
<td>1699.1 ± 137.3</td>
</tr>
<tr>
<td>upper hypertrophic zone</td>
<td>448.6 ± 38.9</td>
<td>289.6 ± 55.0</td>
<td>712.2 ± 107.1</td>
</tr>
<tr>
<td>lower hypertrophic zone</td>
<td>295.9 ± 34.2</td>
<td>207.3 ± 41.8</td>
<td>638.4 ± 90.8</td>
</tr>
</tbody>
</table>

* Data are given as mean ± SD. Abbreviations as in Table 1.
* b P < 0.05 compared with SAL.
* c P < 0.05 compared with SPF.

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*Figure 3.* 5-Bromo-2’-deoxy-uridine (BrdU)-labeled cell proliferation in the proximal tibial growth plate of SAL (a) and NX (b) rats. Sections counterstained with hematoxylin. Magnification, ×210.

*Figure 4.* Sections through the lower growth plate and upper metaphyseal portion of SAL (a) and NX (b and c) rats. Growth plate from NX rats is more irregular and presents chondrocytes with small size appearing organized into vertical columns of flattened cells resembling those of the proliferative zone (arrows). Magnification: ×170 in a and b; ×450 in c.
columns every day (one chondrocyte every 3.4 to 3 h) in SPF and SAL rats, whereas this turnover fell to approximately five cells per day (one chondrocyte every 4.8 h) in the NX group. This decrease in cell turnover was accompanied by a longer duration of the hypertrophic phase, which was extended from, approximately, 33 h in both control groups to nearly 90 h in NX rats. Finally, the lineal velocity of advance of chondrocytes in the columns was significantly decreased in NX rats when compared with control animals.

Discussion

Elongation of long bones is the result of the interplay of two coupled processes: continual and vectorial production of cartilage by the growth plate and replacement of cartilage by bone tissue at the epiphyseal/metaphyseal interface in a process called ossification. A strict coordination between the processes of cartilage enlargement, cartilage resorption, and osseous tissue formation at the metaphyseal end is required for normal bone growth. The results presented here indicate that both production of cartilage and ossification were significantly slowed down in uremic rats. Furthermore, the two processes were differentially depressed in the sense that cartilage resorption/bone deposition was affected to a higher degree than cartilage formation. This led to a disequilibrium that resulted in an increased growth plate height as a result of accumulation of cartilage at the hypertrophic zone. This finding cannot be attributed to the nutritional deficit associated with renal failure since the growth plates of SPF rats were not substantially
different from those of SAL animals and, by contrast, growth plates of SPF and NX rats differed markedly.

Earlier studies analyzing the height of epiphyseal growth plates in uremic rats have yielded no uniform results, since, compared with control animals, increased (20,26), decreased (22,32), and unchanged (23–25) height has been reported. This discrepancy may be explained by two factors. First, a systematic random-sampling method is necessary to obtain unbiased measurements, because growth plate is a somewhat twisted disc whose height shows perceptible regional differences (29). This is especially important in the presence of uremia, because the borderline between the distal end of cartilage and the proximal end of bone in nephrectomized rats is rather ill-defined. The degree of renal failure obtained in the different studies may also play a role. Unpublished data from our group indicate that growth plate modifications described in the present report are not found in mildly uremic rats. It is of note that a severe degree of uremia is needed to induce sustained growth failure in subtotal nephrectomized rats. Otherwise, growth retardation is rather due to the period of acute renal failure that follows the second stage nephrectomy than to chronic uremia itself (33). Finally, another source of variation might be the time period of nephrectomy, since it varies in the different studies.

It is also of note that the only measurement of growth plate height provides little information on how a disease is interfering with the metabolism of growth cartilage, and interpretation of this finding may be misleading. In the above-quoted report, Mehls et al. (20) stated that the increased height of tibial growth plate of uremic rats was reminiscent of vitamin D deficiency. However, in vitamin D-deficient rickets, the chondrocytes of the hypertrophic zone have been described to be characteristicly much bigger than those of aged-matched control animals (34), whereas the hypertrophic chondrocyte volume of the NX rats presented here was significantly reduced in relation to the two groups of sham-operated rats with normal renal function (Table 3).

The rate and extent of growth for a given growth plate is determined by a combination of chondrocyte proliferation, matrix synthesis, and cell-controlled phenotype modulation (chondrocytic hypertrophy) (21). The relative contribution of each of these processes to longitudinal bone growth is not well established, since it may vary significantly at different ages and in different species (35). Chondrocyte proliferation has been classically considered as the major modulator of growth (36,37). However, in some disorders, growth rate may be severely impaired in the presence of normal cell proliferation at the growth plate (28,38). Furthermore, the division rates of proliferating chondrocytes in all cartilage plates of a given animal have been found to be fairly constant (39–41). In addition, a positive correlation between the size of chondrocytes in the terminal hypertrophic zone and longitudinal growth rate has been demonstrated in both physiologic and pathologic conditions (27,28,38,42). Thus, it is now generally considered that chondrocyte proliferation tends to be preserved even in pathologic situations and that modifications of longitudinal growth rate are determined mainly by changes in shape and volume during chondrocyte hypertrophy (22,43). In agreement with these findings, retardation of growth in our uremic rats was associated with a significant decrease of both cell height and cell volume at the distal hypertrophic zone (Table 3). Matrix volume per cell or cell proliferation did not significantly change. Thus, it may be concluded that chronic renal failure caused a profound alteration of the chondrocyte hypertrophy process.

Cell size and shape are generally maintained and modulated by components of the cytoskeletal system. However, it has been reported that chondrocyte shape and volume are highly dependent on the composition of the extracellular matrix, which despite being elastic and compressible is also stiff, and thus provides a firm supportive coat around the chondrocyte (27,44). As a result, changes in cell shape and volume during hypertrophy are normally coupled to degradative and synthetic processes in the surrounding matrix (45,46). In our NX rats, the matrix volume per chondrocyte was not significantly modified when compared with control rats. However, the velocity of advance of chondrocytes at the hypertrophic zone of the growth plate was slowed down in uremic rats (Table 4). Such movement is not passive but requires vectorial degradation of matrix at the metaphyseal cell pole and resynthesis at the epiphyseal cell pole. Thus, our results indicate that renal failure impairs matrix remodeling at the hypertrophic zone of growth cartilage.

Our study extensively describes the modifications induced by uremia at the long bone growth plate level and reveals that increased height of growth plate results from a disequilibrium between cartilage formation and cartilage resorption, which is related to significant changes in the dynamics and morphology of chondrocytes. Unfortunately, our study does not provide any insight into the mechanisms underlying the defective maturation process of growth plate chondrocytes found in uremic rats. It is tempting to speculate that substances that modulate cell hypertrophy and whose metabolism is altered in uremia might be responsible for this effect. Very little is known about the influence of uremic state on the effect of the several endocrine, paracrine, and autocrine factors that act differentially on chondrocytes according to their stage of differentiation (21,47,48–50). Low levels of mRNA insulin-like growth factor-1 in chondrocytes of proliferative and hypertrophic zones of the growth plate (24) and resistance to GH and insulin-like growth factor-1 in primary cultures of epiphyseal chondrocytes (51) have been reported in uremic rats. Further investigations are clearly needed to clarify how chronic renal failure interferes with the activity of the epiphyseal growth plate and the normal maturation of their chondrocytes.

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References